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BEHAVIORAL ECOLOGY OF INVASIVE ANT SPECIES IN HAWAII

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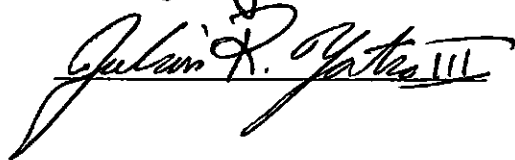
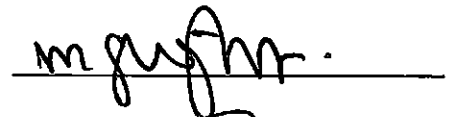
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ABSTRACT

The ant species in Hawai'i are all non-native, creating a unique and interesting environment in which to study their invasion biology. In regards to which species will become dominant in the invaded habitat, I proposed that a predictive tool would be useful to protect ecosystems from destructive alien species. Agonistic laboratory bioassays will examine the interspecific aggression of selected ant species in order to assess mechanisms that may lead to species dominance in the field.

I have examined interactions between ants and one termite species, five ant species both dominant and subdominant, and four exclusively dominant ant species. I have related the results found in the agonistic laboratory assays to field surveys that were conducted regarding ant species abundance. The results indicate that there are parallels between aggressive species in the laboratory assays and high abundance in the surveyed field sites. In conclusion, agonistic assays provide a generally realistic picture of ant species dominance in the field.

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INTRODUCTION

The Hawai'ian Islands are one of the few ecosystems in the world that lack native ants. Approximately 45 ant species have invaded Hawai'i, and about half of these are prominent in urban, agricultural and natural environments (Reimer 1994, Krushelnycky 2005). The ant assemblages in Hawai'i are all synthetic, creating a unique and interesting environment in which to study invasion biology with respect to establishment, ecological interactions, and impacts. In regards to which species will become dominant in the invaded habitat, I proposed that a predictive tool would be useful to protect ecosystems from destructive alien species.

Chapter one examines the interspecific interactions between termites and ants through evaluations of the aggressive behavior elicited by these invasive social insects. The agonistic interactions of these four selected ant species with the Formosan subterranean termite (an earlier invader) may help to evaluate the invasive potential of these ants in their new habitat. This is an opportunity to assess levels of aggressive or defensive behavior shown by the four ant species towards the termite that may otherwise be difficult to observe in the field. In turn, the behavior demonstrated by *C. formosanus* towards the ants may enable us to assess any past ecological relationships with these ant species. The levels of aggression from each may also illuminate which species pose more serious threats to Hawai'i's ecosystem.

In chapter two, both abundant and relatively rare ant species were selected for study. Our goal was to elucidate the invasive biology of these ants and gain insight into the community structure in Hawai'i. Combining individual and group (colony level) assays provides a comprehensive method to test ant species agonism (Moller 1996, Retana and

Cerda 1995). We addressed the following key questions: how dominant and subdominant species interact in terms of aggression when paired individually and in larger groups; the differences in aggression shown between the two types of assays; and how agonistic behavior may relate to the status of being a dominant and subdominant species in Hawai'i.

Similarly, in chapter three, agonistic interactions among ant species was assessed. Unlike the previous chapter which used both dominant and subdominant species, this chapter assesses behavioral interactions among exclusively dominant ant species in order to evaluate whether avoidance or aggression are primary mechanisms leading to their success. We addressed the following key questions: how typical dominant species in Hawai'i interact with other dominant species when paired individually or in groups; and how agonistic behavior may relate to determining dominant species in Hawai'i.

Chapter four explores the correlation between aggressive behavior in agonistic laboratory assays and abundance and dominance in the field. Field surveys were performed at the same locations where, approximately one year earlier, ant species had been collected for behavioral bioassays. I propose that the species with highest levels of aggression would continue to be the most abundant at each site from which they were collected in the previous year.

CHAPTER 1

Agonistic interactions of four ant species occurring in Hawai'i with *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

Introduction

Of the 44 ant species known to occur in Hawai'i (Wilson and Taylor 1967, Reimer et al. 1990, Krushelnycky 2005), there has been limited research on these invasive species; *Leptogenys falcigera* Roger (sickle-tooth ant), *Camponotus variegatus* Smith (Hawai'ian carpenter ant), *Anoplolepis gracilipes* Smith (long-legged ant), and *Wasmannia auropunctata* Roger (little fire ant); and none on their interactions with the subterranean termite *Coptotermes formosanus* Shiraki. All of the ant species found in the Hawai'ian Islands are invasive (Wilson and Taylor 1967, Reimer 1994), and due to the lack of this major insect family, Formicidae, it creates a unique ecosystem in which to study invasion biology. Several reports have shown that ants have devastating impacts to the native flora and fauna of ecosystems they invade (Holway 1998, Holway et al. 2002, Le Breton 2004, Krushelnycky 2005, Reimer 1994). Many factors contribute to ants being successful colonizers including low intraspecific aggression (unicolonial nests), high interspecific aggression, and mutualistic relationships with Hemipteran insects (Holway et al. 2002; O'Dowd et al 2003; Helms and Vinson 2003). This study focuses on competitive characteristics that may lead to the success of invasive ant species in Hawai'i.

We chose to test the interactions between termites and ants in which to assess aggressive behavior elicited by these invasive social insects. The four selected ant species and their agonistic interactions with the Formosan subterranean termite (an earlier invader) will help to evaluate the invasive potential of these ants to their new habitat.

This is an opportunity to assess levels of aggressive or defensive behavior shown by the four ant species towards the termite that may otherwise not be observed in the field. And the behavior demonstrated by *C. formosanus* towards the ants may enable us to assess ecological relationships with these ant species. The levels of aggression from each may expose which species pose a threat to Hawai'i's ecosystem.

We selected both dominant and subdominant ant species for this study. Dominance may reflect the extent of time since initial introduction to Hawai'i. *Leptogenys falcigera* is an old world species and one of the original ants to colonize the Hawai'ian Islands in the late 1800's (Smith 1879). This nomadic ant from the Ponerini tribe forms small colonies (50-100 workers) and has ergatoid (worker-like) queens. It feeds mainly on isopods and inhabits dry soil and tree cavities. *Camponotus variegatus* is native to Southeastern Asia and is also documented as one of the pioneering ant species to arrive in the Hawai'ian Islands (Smith 1879). *Camponotus variegatus* also produces smaller colonies with approximately 100 workers, soldiers and one queen. This ant will feed on sugar based foods as well as dead insects. *Anoplolepis gracilipes* is speculated to have originated from West Africa, India or China (Wetterer 2005). This species has a widespread distribution on all the major Hawai'ian Islands. *Anoplolepis gracilipes* is known as a tramp ant and is on the list of "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Specialist Group (ISSG 2006). *Wasmannia auropunctata*, originally from South America, is the most recent invader to Hawai'i and has been recorded on the Island of Hawai'i and in a single locality on Kauai'i. *Wasmannia auropunctata* is a known tramp ant species and is also listed by ISSG (2006). Originally

from China, the subterranean termite, *C. formosanus* is Hawai'i's most economically damaging insect pest (Yates & Tamashiro 1990) occurring throughout the Islands.

Interestingly *L. falcigera* and *C. variegatus* were first recorded in Hawai'i in the late 1800s and *A. gracilipes* and *W. auropunctata* were discovered more recently in 1952 and 1999 respectively (Krushelnycky 2005, Conant and Hirayama 2000, Smith 1879, Zimmerman 1953). Perhaps the dominance of *A. gracilipes* and *W. auropunctata* is related to their more recent introduction into Hawai'i, and the ability to out compete *L. falcigera* and *C. variegatus*. As ants are major predators of termites (Deligne et al 1981), we conducted experiments to investigate potential agonistic interactions among these species. We tested the hypothesis that recently arrived ant species demonstrated higher aggressive behavior than long- established species. The agonistic assays also allow us to evaluate the level of aggression that each species has in paired trials. The assays are the first experiments in a larger study to assess the competitive nature of invasive ant species in Hawai'i.

Methods and Materials

Termite and ant collection

Formosan subterranean termites were collected on the Manoa Campus of the University of Hawai'i using a trapping technique designed by Tamashiro et al. (1973). The termites are lured to wooden stake placed about ten centimeters below grade and is enclosed by a wooden trap; therefore making them easily assessable for collection and experimentation. The termites were sampled from three distinct colonies on the campus.

In order to minimize stress, termites were extracted from the traps and used the same day of experimentation. The ant species *L. falcigera* and *C. variegatus* were collected from the soil or within the branches or trunks of trees at an experimental farm in Waimanalo, Oahu, Hawai'i. *Anoplolepis gracilipes* was collected from Tantalus Forest Reserve above Honolulu in Hawai'i. The ants were carefully extracted from original nests and reared in the laboratory at an average 25°C. They were fed on a combination of 25% sucrose aqueous solution, peanut butter, sweet corn, tuna fish in oil and dead insects. *Leptogenys falcigera*'s main diet was 25% sucrose solution and isopods. *Wasmannia auropunctata* was collected on the Island of Hawai'i in the Puna district from the soil of a flower pot and all foraging ants were assumed to come from the same colony. Since this species could not be moved between islands due to quarantine restrictions, agonistic assays were conducted on the Island of Hawai'i with termites from the Manoa campus.

Experimental design

Agonistic bioassays between the ants and termites were conducted in a 60x15mm Petri dish, following the methods of Cornelius and Grace (1994). A termite soldier was gently placed in a Petri dish coated with fluon. Once the termite began to calmly move around the Petri dish a worker from one of the four ant species was then added to the arena. Responses were observed and recorded for both the ants and the termites as follows: avoidance (-1), no response (0), or aggression (1). Avoidance behavior would be scored if the termite or ant made physical contact and then immediately went in the opposite direction. No response would be scored when no other visible behavior after physical contact between the termite and ant occurred. Aggressive behavior would be scored when biting, mandibles open, or any other attacking act would be elicited after

initial physical contact. The first two interactions were observed for each exposure and the highest scored response was recorded. To test consistency within a species, we used three separate colonies from all species. The blocking design consisted of one of the three ant colonies from each species paired with one of the three termite colonies. The protocol included 20 replications for each pairing.

Statistical Analysis

To test overall species behavior using GLM Procedure (SAS 9.1), the percent mean response from the ant and the termite were calculated and transformed to arcsine square root. This analysis compared behavior among all species. To compare behavior within each species for each pairing we conducted additional analysis using percent means and transformed to arcsine square root in a GLM Procedure (SAS 9.1). Means for both analyses were separated with the Ryan- Einot- Gabriel-Welsch multiple Range Test.

Results

The results of the overall behavioral analysis from the four ant species showed that *W. auropunctata* demonstrated a significantly higher “no response” ($p = 0.0146$, Table 1.1) and “aggressive” ($p = 0.0053$, Table 1.1) behavior towards *C. formosanus* in comparison to the other ant species. There was no significant difference in comparison of *L. falcigera*, *C. variegatus*, *A. gracilipes* overall behavioral response towards *C. formosanus* (Table 1.1). *Coptotermes formosanus* significantly showed overall lowest average aggressive response towards *W. auropunctata* in comparison to *L. falcigera* and *C. variegatus* ($p = 0.0410$, Table 1.2). Figure 1.1 outlines the distinction in behavior from *W. auropunctata* and the other ant species towards *C. formosanus*. This figure also

illustrates the distinction in behavior from *C. formosanus* towards *W. auropunctata* than to the other three ant species.

Table 1.3 shows the separated behavior and analysis of aggression within each pair of species. The trials with *L. falcigera*, *A. gracilipes*, and *C. variegatus* in opposition to *C. formosanus* showed that “no response” was the most frequently observed behavior from the ants perspective ($p > 0.0001$, Table 1.3). *Coptotermes formosanus* elicited a significant greater frequency of “aggression” behavior towards both *L. falcigera* and *C. variegatus* (respectively; $p = 0.0118$, $p = 0.0173$, Table 1.3). In the case of *C. formosanus* and *A. gracilipes*, the termite was observed to “avoid” more frequently in the trials ($p = 0.0011$, Table 1.3). In the case of *W. auropunctata* and *C. formosanus*, *W. auropunctata* was observed to have the highest aggressive response towards *C. formosanus* ($p = 0.0003$, Table 3) and *C. formosanus* showed a higher “no response” behavior towards *W. auropunctata* ($p = 0.005$, Table 1.3).

Discussion

Previous study conducted by Cornelius and Grace (1994) on the agonistic interactions of termites and ants reported that the termites showed a greater proportion of aggressive behavior towards the ants. This aggression elicited in the Cornelius and Grace (1994) assays is suggested to be a defensive trait of termite soldiers evolved in response to predation by ants (Deligne et al. (1981). Our analysis of the interactions between *L. falcigera*, *C. variegatus* and *A. gracilipes* versus *C. formosanus* showed a pattern that is consistent with previous agonistic behavioral studies using ants and termites. The

behavior that was observed in these agonistic assays were primarily aggressive behavior (i.e. open mandibles, biting and attacking). These aggressive responses by *C. formosanus* were elicited by all of the ant species except *W. auropunctata*. In fact there was lower aggressive behavior towards *W. auropunctata* and the highest level of non-responsive behavior from the termite in all trials. The behavior shown by the ants towards *C. formosanus* the majority of the time was non-responsive from *L. falcigera*, *C. variegatus*, and *A. gracilipes*. *Wasmannia auropunctata* was unique from the other ant species in showing much higher levels of aggression towards *C. formosanus* (Figure 1.1).

Studies of invasion biology have shown a pattern of high interspecific aggression among introduced ants towards other ants and arthropod species (Passera 1994, Holway and Suarez 1999, Morrison 2000). In our laboratory assays this high level of aggression is certainly shown by *W. auropunctata*, the most recent invader in Hawai'i. Previous studies of invasive ants have described that the majority of aggressive behaviors are shown by cosmopolitan tramp ants. Tramp ants are characterized as having decreased or lack of intraspecific aggression and increased interspecific aggression; and they often form large multi-colonial nests (Holway 1998, Suarez et al 1999, Holway et al., 2002, Tsutsui and Suarez, 2003). Tsutsui et al. (2000) suggests that this decreased intraspecific aggression and increased interspecific aggression in introduced species is due to a reduction in genetic variability between colonies resulting in a propensity to recognize individuals from different colonies as nestmates (Tsutsui et al. 2003). This phenomenon has been explored in recent studies by Le Breton et al. (2004) that show *W. auropunctata* having higher interspecific aggression in its introduced habitat than in its native range. These authors also show that *W. auropunctata* has lower intraspecific aggression in

introduced habitats than in its native range. Several authors believe that this shift in behavior is one mechanism that lends to the successful establishment of this species in a new environment and hence the status of a cosmopolitan tramp ant (Holway and Suarez 1999, Passera 1994 Holldobler and Wilson 1977).

Although *A. gracilipes* is noted to have polygynous colonies in introduced habitats (Abbott 2005), in our assays this species did not show the same levels of aggression as *W. auropunctata*. Perhaps this discrepancy in behavior between these two species may be attributed to the time of introduction into Hawai'i. The aggressive behavior towards *C. formosanus* may be due to no history of cohabitation in Hawai'i between *W. auropunctata* and *C. formosanus* resulting in heightened aggression. And perhaps the other ant species have stabilized their behavior to a non aggressive response towards the termite having inhabited Hawai'i longer. In a review publication on the ecology, policy and management of ants in Hawai'i, Krushelnycky et al. (2005) have proposed that the current ant assemblage is approaching equilibrium with the exception of turnover in dominant species. *Anoplolepis gracilipes* and *W. auropunctata* are both considered to be highly invasive species (ISSG 2006) and perhaps *W. auropunctata* is the new dominant species taking the "place" of *A. gracilipes*. These sets of agonistic trials with termites and ants have shown that the newest invader is the most aggressive and defensive whereas the "older" established species may have "coevolved" to show less aggression. We propose that future research on behavioral interaction of among ants in Hawai'i with a wider range of behaviors to choose from will further support our investigation into the invasive biology of ant fauna invading Hawai'i.

CHAPTER 2

Agonistic interactions among five invasive ant species from two habitats on Oahu, Hawai'i

Introduction

The Hawai'ian Islands are the most isolated archipelago in the world. These islands have a complex assemblage of plants and endemic arthropods that evolved without any Formicidae predators (Wilson and Taylor 1967). A small subset of the 45 exotic ant species that have been successfully established in Hawai'i are considered to be tramp ants (i.e. polygynous, generalist, large colony size) (Holway et al. 2002). With respect to which species will become dominant in the invaded habitat, previous research has proposed that a predictive tool would be useful to protect ecosystems from destructive alien species (Holway et al. 1998, Holway and Suarez 1999, Holway et al. 2002, Moller 1996, Morrison 2000). We suggest that agonism among invasive ant species in Hawai'i can be used as a tool in predicting which species is the most dominant with the potential to have greater ecological effects on native arthropods and plants.

A study conducted by Morrison (1996) in the Society Islands, French Polynesia also examined the invasive biology of ants. These islands are similar to the Hawai'ian Islands, in that they also lacked native ants. Morrison's research assessed the community structure of dominant and subdominant ant species, particularly how non-native ant communities establish when introduced to an insular ecosystem. Morrison (1996) found that one mechanism leading to the dominance of invasive *Solenopsis invicta* is its superior ability for colony-level competition (high aggression) and cohabitation with

subdominant ant species. The relationships among dominant and subdominant ant species have been studied in a number of habitats, including cocoa plantations in Brazil and Papua New Guinea (Room 1975, Majer et al 1994). Many of the ant species studied have invaded Hawai'i (i.e. *A. gracilipes*, *W. auropunctata*, *P. megacephala*), creating an opportunity to explore the community structure of invasive dominant and subdominant ant species and determine if it is consistent with patterns demonstrated elsewhere.

Interspecific competition is one mechanism that results in aggression between species. The resulting contest will often result in one species having dominance over another in a particular habitat (Holldobler and Wilson 1990). Holway et al. (2002) described the general characteristics of invasive ants, and aggression is a significant characteristic of successful species. Holway et al. (2002) and Holldobler and Wilson (1990) report additional characteristics that are inclusive but not exclusive for all established introduced ants. These characteristics include polygynous colonies, habitat generalists, and large colony size. These characteristics are shown to be important traits for successful dominant ant species, but can be confounding when studying tramp ant species. As mentioned above, tramp ants tend to have low intraspecific aggression (leading to large colonies with multiple queens) which can then lead to overlapping nests or supercolonies (Passera 1994, Holldobler and Wilson 1977), thus making it difficult to differentiate distinct colonies in the field. To avoid sampling discrepancies in the field between multicolonial nests, laboratory bioassays assessing aggression among different species should provide an accurate method to test mechanisms that lead to dominance.

Agonistic behavior, as defined by King (1973), "includes all behaviors associated with the contest or struggle between individuals." For agonistic trials in the laboratory, we

sampled two different habitats on Oahu, Hawai'i, in order to compare interspecific interactions of ants within and among the communities. We selected both abundant and rare ant species for this study. *Leptogenys falcigera* is an Old World species originating from Africa and one of the original ants to colonize the Hawai'ian Islands in the late 1800's (Smith 1879). This nomadic ponerine ant forms small colonies (50-100 workers), has ergatoid (worker-like) queens, feeds mainly on isopods, and inhabits dry soil and tree cavities (Bolton 1973). *Camponotus variegatus* is native to Southeastern Asia and is also documented as one of the pioneering ant species in the Hawai'ian Islands (Smith 1879). This ant produces colonies with approximately 100 workers, soldiers, one queen, and feeds on sugar based food and dead insects. The exact origin of *A. gracilipes* is not known but is speculated to originate from West Africa, India or China (Wetterer 2005). This species has a widespread distribution on all the major Hawai'ian Islands (Reimer et al. 1990). *Anoplolepis gracilipes* is known as a tramp ant and is on the list of "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Specialist Group (ISSG 2006). *Ochetellus glaber* was first recorded on the Hawai'ian Islands in 1977 and originated from Australia and Japan (Beardsley 1980). This species is abundant on Kaua'i, Oahu, Maui and the Island of Hawai'i (Reimer et al. 1990). *Pheidole megacephala* is a tramp ant and is also listed on "100 of the World's Worst Invasive Alien Species" (ISSG 2006). This species was first discovered in Hawai'i in 1879 (Smith 1879) and originates from southern Africa.

Our goal was to elucidate the invasive biology of the ants and perhaps gain insight into the community structure of these species in Hawai'i. Combining individual and colony level assays provides a comprehensive method to test ant species agonism (Moller

1996, Retana and Cerda 1995). We addressed the following key-questions: how dominant and subdominant species interact in terms of aggression when paired individually and in larger groups; the differences in aggression between the two types of assays; and how agonistic behavior may relate to the status of being a dominant and subdominant species in Hawai'i.

Methods and Materials

Collection of ants

Ants were collected at two sites on Oahu, Hawai'i that differed in elevation and temperature. The first site, Tantalus Forest Reserve above Honolulu, Hawai'i is 634 meters above sea level with an average yearly rainfall of 266.25 cm and 20.3 °C. The other site at Waimanalo Experimental Research Station of the University of Hawai'i is on the windward side of the island of Oahu and is 18m above sea level with an average yearly rainfall of 110.76cm and an average temperature of 23.8 °C. We chose these two habitats because of their environmental and ant species variation in order to assess agonism among ant species that otherwise might not interact in the field.

Four ant species, *L. falcigera*, *C. variegatus*, *P. megacephala*, and *O. glaber* were collected from the Waimanalo Experimental Research Station. The fifth species, *A. gracilipes*, was collected from Tantalus. The ants were kept in the laboratory at 25 °C and fed 25% sucrose solution, peanut butter, sweet corn, tuna fish in oil and dead insects. *Leptogenys falcigera* mainly fed on 25% sucrose solution and live isopods collected from the field.

Laboratory Bioassays

In laboratory bioassays where individuals of each species were paired, interactions were recorded using a rating system with a range of nine different behaviors. These assays show agonistic behavior on an individual level and can be taken as a measure of the innate aggression of each species. The second set of experiments were group agonistic assays, where groups of ten individuals from each species were paired. These experiments are more representative of agonistic interactions at the colony level.

In the individual assays, an individual of one species was gently placed with a wooden stick, or aspirated, into a 60x15 mm Petri dish coated with fluon near the rim to prevent escape. The ant was given 1-2 min to acclimate before another individual of one of the other four ant species was likewise transferred to the opposite end of the dish. We recorded any behavioral interactions for ten minutes, following the methods of Retana and Cerda (1995) with a nine-point rating system graded from offensive to defensive behavior as follows: bite, gaster flex, attack, mandibles open, indifference, mutual investigation, escape, being attacked, and being bitten. Each response was noted and totaled numerically, with five replicates for each species pairing. Behavioral interactions were recorded from the perspective of each of the two ant species in each trial.

Group assays were evaluated on the basis of ant mortality, with the assumption that aggressive interactions lead to mortality. Ten individuals of each species were paired in 400ml glass containers coated with fluon around the edges. Paired species were left for three hours, with four replications of each species pairing. Only one replication for pairs with *L. falcigera* was conducted due to small colony size. Controls were initiated at the same time as the two-species pairings, with 20 individuals from the same species placed

in a container for three hours. At the end of the incubation period, survivors were recorded to assess mortality.

Statistical Analysis

Individual assays were analyzed by overall species behavioral response using Chi-square analysis. Specific responses within each pair of species were analyzed by GLM Procedure in SAS. T-tests were used to test mean differences in mortality among pairs in group assays (SAS Institute, 2002-2003). In the individual assays, means were separated with the Tukey's Studentized Range (HSD) Test to determine which behaviors were most frequently observed.

Results and Discussion

Pheidole megacephala and *A. gracilipes* were the two species in the individual assays with the highest number of aggressive responses. Table 2.1 shows the total number of acts for each behavior (i.e. attack, bite, gaster flex, mandibles open, indifference, mutual investigation, escape, being bitten, and being attacked) when each species was paired against each of the other four species. All of the species except *C. variegatus* exhibited significant difference in frequencies of these different behaviors (Table 2.1). The Chi-square values are as follows: *P. megacephala*: $\chi^2 = 146.1$; 2 df. $P < 0.05$, *A. gracilipes* $\chi^2 = 12.7$; 2 df. $P < 0.05$, *O. glaber* $\chi^2 = 33.0$; 2 df, $P < 0.05$, *L. falcigera* $\chi^2 = 326.9$, 2 df, $P < 0.05$. Table 2.2 shows the nine behaviors summarized into three categories: aggression, no response, and defensive; and then comparatively ranked among species. To test statistical difference among pairs we analyzed the mean of the nine behavioral interactions using the general linear models procedure (SAS Institute, 2002-2003). All

pairs except the pairing of *C. variegatus* and *O. glaber* (from the perspective of *C. variegatus*) demonstrated significantly different behavioral responses. For example, *P. megacephala* elicited a significantly different behavior of “mandibles open” than the other eight behaviors. *Ochetellus glaber* showed significantly more “mutual investigation” acts than the other eight behaviors.

The group assays were analyzed by a paired t- test to assess significant levels of survival within each pair. Table 2.3 shows the average survival in all four trials for each species within a pair. Each pair except for *C. variegatus* and *O. glaber* ($P = 0.191$) had significant differences in survival ($P < 0.05$). *Pheidole megacephala* versus *A. gracilipes* showed the highest levels of aggression, in which *P. megacephala* had 0% survival against *A. gracilipes* in all four trials. Similarly, *C. variegatus* versus *P. megacephala* showed high levels of agonism, in which *P. megacephala* suffered 0% survival in three out of the four trials. *Leptogenys falcigera* had the highest average survival rate, with 100% survival in all four trials paired with all five species. Mortality of workers in control trials was negligible with less than 5% mortality.

The most aggressive species in individual assays were *P. megacephala* and *A. gracilipes*, as they ranked first and second, respectively, in the aggression category. *Pheidole megacephala* also ranked last in the no response and defensive categories in comparison to the other four species. *Anoplolepis gracilipes* followed the same pattern as *P. megacephala* in the no response and defensive categories, also showing low levels of no response and defensive behavior compared to the other three species. These data are comparable to qualitative field observations at the time of collection in the spring and fall of 2005, when *P. megacephala* and *A. gracilipes* were observed to be the most common

species in Waimanalo and Tantalus, respectively. The high aggression and low defensive behavior of *P. megacephala* and *A. gracilipes* parallels observation at these two collection sites and other surveys of ant distribution in Hawai'i (Huddleston and Fluker 1968, Reimer and Beardsley 1990, Reimer 1994). The behavioral responses of *L. falcigera* in the individual and group assays were also similar to what was observed in the field at the time of collection. *Leptogenys falcigera* was ranked first in the no response category for the individual assays and had the highest survival rate (100%) for the group assays, which would appear to be consistent with the cryptic nature of this ant in the field. Although little is known of the biology of *L. falcigera*, these studies suggest that the high levels of indifferent behavior shown in individual assays and the highest average survival in group assays may reflect the mechanism that this species uses to survive when competing against more aggressive species.

According to previous distributional surveys of ant species in Hawai'i, *P. megacephala* and *A. gracilipes* tend to not overlap in their range (Fluker and Beardsley 1970, Huddleston and Fluker 1968). The other three species that were sampled (*C. variegatus*, *O. glaber*, and *L. falcigera*) were found in the same habitat as *P. megacephala* at Waimanalo, but not at the Tantalus location. Perhaps the higher levels of aggression of *P. megacephala* are due to the exposure to the other three species in Waimanalo; and thus *A. gracilipes* was not as aggressive due to lack of experience with the other species. In the case of invasive ants and endemic spiders in Hawai'i, Gillespie (1999) has shown how novel associations among these arthropods have led to a decrease in the native spider populations, because of lack of defense against the ants. Novel associations of *C. variegatus*, *O. glaber*, and *L. falcigera* with *A. gracilipes* in our

laboratory assays may explain why these pairings resulted in lower levels of agonism due to lack of defense.

Based upon species abundance observed in the field, there were distinct dominant and subdominant species at the time of collection. As mentioned above, previous studies have examined the community structure of dominant and subdominant ant species that are commonly found in the same habitat (Morrison 1996, Morrison 2000, Room 1975, Majer et al. 1994). One mechanism permitting co-occurrence of dominant and subdominant species may be temporal or spatial separation in foraging or nesting habits (Suarez et al 2005). For example, *P. megacephala* is primarily a diurnal ground forager and *C. variegatus* is a nocturnal ground forager (Huddleston and Fluker 1968, Reimer and Beardsley 1990). *Ochetellus glaber* is an arboreal species, and *L. falcigera* is both a ground and arboreal species with infrequent occurrences and cryptic foraging strategies. These distinctions in their nesting and foraging habits may allow these four ant species to coexist in the same environment. Although these assemblages of ant species co-occur in the field, they still demonstrate aggressive behavior when forced to interact in laboratory assays.

The group assay results were based on mortality as an indicator of aggression levels. The consequence of high agonism between pairs was the death of one or both species. All *P. megacephala* group trials resulted in less than 100% survival of the initial ten individuals in the trials. For example, *P. megacephala* survival varied from an average of 50% in all trials paired with *O. glaber* to total mortality of all individuals in all the trials paired against *A. gracilipes*. The results show that *P. megacephala* is indeed an aggressive species but is not necessarily successful in combat, which suggests that there

are other mechanisms aside from aggression that *P. megacephala* is using in the field to become a dominant species. Large colony size is one popular theory for the success and dominance of ant species (Walters and Mackay 2005), suggesting that perhaps if larger numbers of *P. megacephala* individuals were included in the group assays, more individuals would survive. Similar agonistic assays were conducted by Fluker and Beardsley (1970) where *P. megacephala* resulted in high mortality when paired against *Linepithema humile* (Mayer) and *A. gracilipes*. Fluker and Beardsley (1970) conducted additional assays where more individuals were included in trials paired with *L. humile* resulting in higher survival. Because *P. megacephala* was ranked first in aggression in individual assays and was highly agonistic in the group assays, the low survival of this species is perhaps at least partially due to the low number of individuals in the assays.

The results of our individual and group assays parallel our qualitative observations of abundance and dominance in the field in that *P. megacephala* and *A. gracilipes* had the highest levels of aggression in individual assays and high agonism in group assays. As mentioned by Holway and Suarez (1999), Holway et al. (1998), and Morrison (2000) in their studies of behavioral mechanisms to assess ant invasions, agonistic assays can be important tools in predicting which species are dominant and potentially harmful to invaded habitats.

CHAPTER 3

Assessment of agonism among tramp ant species *Anoplolepis gracilipes* (Smith), *Pheidole megacephala* (Fabricius), *Linepithema humile* (Mayr), and *Wasmannia auropunctata* (Roger)

Introduction

The Hawai'ian archipelago is the most isolated group of islands. Hawai'i has a high proportion of endemic arthropods and plants, but the ca. 45 ant species present in Hawai'i are all introduced (Wilson and Taylor 1967, Reimer et al. 1990). The majority of the ant species that have been successfully established in Hawai'i are considered to be tramp ants (i.e. polygynous, generalist, and large colony size). Previous researchers on invasive biology of ants have proposed a number of theories attempting to explain species dominance, including heightened interspecific aggression, lessened intraspecific aggression, recruitment efficiency, and large colony size (Wilson 1971, Holldobler and Wilson 1990, Holway et al. 2002, Walters and Mackay 2005). The term "tramp ant" (Passera 1994) describes species that are consistent with the above mentioned theories and thus the likelihood of the successful establishment in introduced habitats.

Interspecific competition is one mechanism that results in aggression between species. This behavior will often result in one species having dominance over another in a particular habitat (Holldobler and Wilson 1990). Studies by Holway et al. (2002) show the general characteristics of invasive ants, and aggression is a significant characteristic of successful species. Holway et al. (2002) and Holldobler and Wilson (1990) report additional characteristics that are inclusive but not exclusive for all established

introduced ants. These characteristics include polygynous colonies, generalist nesting habits, and large colony size. The latter characteristics are shown to be important traits for successful dominant ant species, but can be confounding when studying tramp ant species. As mentioned above, tramp ants tend to have multiple queens leading to low intraspecific aggression (low genetic diversity) which can then create overlapping nests or supercolonies (Passera 1994, Holldobler and Wilson 1977), thus making it difficult to differentiate distinct colonies in the field. To avoid discrepancies in the field because multicolonial nests can be inadvertently sampled, laboratory bioassays assessing aggression among different species should provide an accurate method to examine interspecific interactions. And this reductionist approach enables the researcher to focus on specific behaviors (i.e. aggression, avoidance, and defense) in order to see more clearly what mechanisms are involved (Holway and Suarez 1999).

Unlike previous agonistic assays I conducted using dominant and subdominant invasive species to assess competitive mechanisms that influence community structure in Hawai'i, this study addresses behavioral interactions among exclusively dominant ant species. The ant species we selected, *Anoplolepis gracilipes* (Smith), *Pheidole megacephala* (Fabricius), *Linepithema humile* (Mayr), and *Wasmannia auropunctata* (Roger) are considered to be among the most invasive and destructive species in Hawai'i and in the world (Holway et al. 2002, Reimer 1994, Krushelnycky 2005). As these four species are known to be dominant and highly invasive ants in Hawai'i (Reimer 1994, Krushelnycky 2005), we would like to assess their agonistic behavior when forced to interact to see if avoidance or aggression is the primary mechanism leading to their current status as dominant species.

The following four species are widespread throughout the major Hawai'ian Islands and all are listed in "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Group (ISSG 2006). *Anoplolepis gracilipes*, the long- legged ant, is speculated to originate from West Africa, India or China. This species has a widespread distribution on all the major Hawai'ian Islands and is restricted to rocky nesting sites (Zimmerman 1953, Fluker and Beardsley 1970). *Pheidole megacephala* (big-headed ant) was first discovered in Hawai'i in 1879 (Smith 1879) and prefers areas with light rainfall and elevations below 600m (Fluker and Beardsley 1970). *Linepithema humile*, the Argentine ant, originally from Central and South America also prefers light rainfall and has adapted to higher elevations in Hawai'i of 900m-1800m and was first discovered in Hawai'i in 1940 (Fluker and Beardsley 1970, Zimmerman 1941). *Wasmannia auropunctata*, the little fire ant, is originally from South America, is the most recent invader to Hawai'i (1999) and occurs on the Island of Hawai'i and in a single locality on Kaua'i (Conant and Hirayama 2000).

Using both individual and colony level assays is a comprehensive method to test ant species agonism (Moller 1996, Retana and Cerda 1995). Our goal was to further elucidate the invasive biology of the ants and perhaps gain insight into the behavior leading to their dominance in Hawai'i. We addressed the following key questions: how typical dominant species in Hawai'i interact with other dominant species when paired individually or in groups; and how agonistic behavior may relate to determining dominant species in Hawai'i.

Methods and Materials

Collection of Ants

We collected *L. humile* from Puu Huluhulu on the Island of Hawai'i. This collection site is in a National park protecting a "kipuka", a raised vegetated area surrounded by a younger lava flow. *Wasmannia auropunctata* was collected from Papaiko, a small town outside of Hilo on the Island of Hawai'i. The site was on a small fruit orchard with mangosteen and rambutan trees. Although *P. megacephala* and *A. gracilipes* occur on the Island of Hawai'i, we transported both species from Oahu in order to prevent the spread of *W. auropunctata* and *L. humile* to different locations. *Pheidole megacephala* was collected from the Waimanalo Experiment Station of the University of Hawai'i, on the windward side of the island of Oahu. *Anoplolepis gracilipes* was collected in the Tantalus Forest Reserve above Honolulu, Hawai'i, on the opposite side of the Koolau mountain range from the Waimanalo Station.

Individual laboratory bioassays

Bioassays examining interactions among pairs of species were conducted using a rating system following the methods of Retana and Cerda (1995). The rating system graded offensive to defensive behavior as follows: bite, gaster flex, attack, mandibles open, indifference, mutual investigation, escape, being attacked, and being bitten. These experiments showed agonistic behavior on an individual level. Thus, these assays can define the innate levels of aggression of each particular species.

An individual of one species was gently placed with a wooden stick, or aspirated into a 60x15 mm Petri dish coated with fluon near the rim to prevent escape. The ant was given 1-2 min to acclimate before another individual of the other three ant species was

likewise transferred to the opposite end of the dish. We recorded any behavioral interactions for ten minutes of the paired bioassay. Each response was noted and totaled, with five replicates for each species pairing. Behavioral interactions were recorded respective to each of the ant species in the trials so that primary aggression and stimulated aggression in response to attack (i.e. biting and being bitten) was accounted for each species.

Group laboratory bioassays

Group agonistic assays consisted of ten individuals from each species paired in observation arenas. Additional group assays were conducted that included 10% *P. megacephala* soldier caste in the pairings. These experiments attempt to simulate agonistic interactions on a colony level.

Group assays were evaluated on the basis of ant mortality, assuming that aggressive interactions led to mortality. Ten individuals from each species were placed in 400ml glass containers coated with fluon around the edges. Paired species were left for three hours with four replications of each species pairing. Controls were initiated at the same time as the two-species pairings, with 20 individuals from the same species placed in a container for three hours. The number of living individuals for each species was recorded to assess survival.

Statistical Analysis

Individual assays were analyzed by overall species behavioral response, using Chi-square analysis. Specific responses within each pair of species were analyzed by GLM Procedure in SAS. T-tests were used to test mean difference in survival among pairs in group assays (SAS Institute, 2002-2003). Means were separated with the Tukey's

Studentized Range (HSD) Test to analyze specific behaviors that were significantly more frequent from the other behaviors in individual assays.

Results

Individual assays

Outcomes for total possible behaviors for each of the species interactions are illustrated in Table 3.1, showing significant differences (GLM procedure) among the behaviors for each species. *Pheidole megacephala* showed the highest frequency of aggressive behavior with 89 acts of 'mandibles open' and 23 acts of 'attack,' more than double when compared to the other species. Table 3.3 shows the nine behaviors broken down into three categories of aggression, no response, and defensive behavior and the subsequent rank of each species within these categories compared to each other. According to chi-square analysis, each species could be placed into one of the three categories. *Pheidole megacephala* ranked first in the aggression category and elicited significantly greater frequencies of aggressive behavior ($\chi^2 = 49$, $df = 2$, $P < 0.001$). *Linepithema humile* ranked second in both aggression and defensive category but did not show any particular pattern of behavior towards any of the other species (Table 3.2-3.3). *Pheidole megacephala* did, however, show the highest aggressive behavior towards *A. gracilipes* and second highest towards *W. auropunctata* (Table 3.2). Likewise, *W. auropunctata* showed the highest number of aggressive acts towards *P. megacephala*, and *A. gracilipes* showed the highest number of defensive acts towards *P. megacephala* (Table 3.2). These results show that there is high agonism among *P. megacephala* and *W. auropunctata*, and *P. megacephala* and *A. gracilipes* in individual assays. *Anoplolepis gracilipes* showed

highest number of ‘indifference’, ‘mutual investigation’, and ‘escape’ behavior throughout all pairings ($P < 0.001$) ranking it first in the defensive category and second in the no-responsive category (Table 3.3). *Anoplolepis gracilipes* showed its highest number of non-responsive acts towards *W. auropunctata* and similarly, *W. auropunctata* showed the highest number of non-responsive acts towards *A. gracilipes*. The behavioral interactions among *W. auropunctata* and *A. gracilipes* show low levels of agonism in individual assays suggesting that these two species do not recognize each other as competitors.

Group assays

In assays with no soldiers present, *P. megacephala* suffered the lowest survival compared to all species with an average of 11% survival for all trials (Table 3.4). When paired with *A. gracilipes* and *W. auropunctata*, *P. megacephala* had 0 % survival in all four replications. *Linepithema humile* had the second lowest average survival at 33% with the lowest average survival at 3% when paired with *W. auropunctata* (Table 3.4). *Wasmannia auropunctata* resulted in an average of 49% survival in all pairing with the lowest survival when paired against *L. humile* at 38% (Table 3.4). *Anoplolepis gracilipes* had the highest survival of individuals with an average 77% in all trials (Table 3.4). All species paired with *P. megacephala* with no soldiers present showed a significant difference in mean survival for each pairing ($P < 0.050$). *A. gracilipes* and *L. humile* also showed a significant difference in mean survival of individuals ($P < 0.050$).

The trials that included *P. megacephala* soldiers resulted in approximately the same average survival for *A. gracilipes* and *W. auropunctata* (Table 3.4-3.5), but drastically changed the average survival for *P. megacephala* and *L. humile* (Table 3.4-3.5). When

soldiers were present, *P. megacephala* had the second lowest average survival and *L. humile* suffered the lowest average survival (Table 3.5). *Anoplolepis gracilipes* consistently had the highest survival with an average of 83% and 77% in trials with and without *P. megacephala* soldiers respectively (Table 3.4-3.5). *Wasmannia auropunctata* also showed a relatively constant average survival of 42% to 49% with and without *P. megacephala* soldiers respectively (Table 3.4-3.5). There was no significant difference in mean survival between any pairs when *P. megacephala* soldiers were present (Table 3.5).

Discussion

Our data from both the individual and groups assays are similar to the results from Fluker and Beardsley (1970) study of the agonistic interactions among *P. megacephala*, *L. humile* and *A. gracilipes*. Throughout all the assays we conducted, *P. megacephala* was the most aggressive species where it consistently initiated attack in all paired trials. In agreement with the results from Fluker and Beardsley (1970), this species was extremely aggressive, but was not entirely successful in killing its opponents. When *P. megacephala* soldiers were added to group trials, this species became “stronger” reducing the average survival rate of *W. auropunctata* and *L. humile*. Clearly, *P. megacephala* soldiers assisted this species in combat against the other two species, but *P. megacephala* still experienced high mortality at an average at 64%. The Fluker and Beardsley (1970) assays showed that an increase in *P. megacephala* individuals in agonistic trials did indeed result in increased success against *L. humile*. The agonistic assays in this study

and in Fluker and Beardsley (1970) suggest that the success of this species being a dominant in the field is in part due to its aggressive behavior in conjunction with its large colony size.

Linepithema humile ranked second in the defensive and aggression category, demonstrating high agonism in individual trials, but in group trials it resulted in the lowest average survival among all species with *P. megacephala* soldiers present. In these laboratory assays, *L. humile* exhibited similar overall results compared to *P. megacephala* in that it is an aggressive species, but not very successful in group combat. The major difference between the behavior of *L. humile* and *P. megacephala* is that *L. humile* is less inclined to initiate attack, but when provoked will defend itself (Fluker and Beardsley 1970). In previous studies with *L. humile*, it has been shown to owe much of its invasive success to its large colony size (Walter and Mackay 2005). This may be the driving mechanism that allows this species to be dominant in the field while its aggressive behavior is only complementary to its success.

Pheidole megacephala and *L. humile* were the most aggressive species in individual assays, both eliciting their most aggressive behavior towards *A. gracilipes*. Not only was *A. gracilipes* a threat to *P. megacephala* and *L. humile* in individual assays, this species consistently had the highest average survival in the group assays. The same results appeared in the Fluker and Beardsley (1970) study where *A. gracilipes* was also successful in group combat. Fluker and Beardsley (1970) related this success to its toxic defensive sprays also shown in this study with 13 counts of gaster flex, the highest amount out of all other species. In addition to the high levels of agonism in this study, the success of this species invasion is confirmed in previous research on Christmas Island

and Bird Island, where *A. gracilipes* abundance and dominance have shown to have negative effects on native vertebrate populations and in some cases causing local exclusion (Abbott 2005, Hill et al. 2003). Although highly agonistic, *A. gracilipes* is limited in its expansion on the Hawai'ian Islands due to the lack of resources, e.g. rocky nesting sites and no less, outcompetition by other dominant species. (Wilson and Taylor 1967, Fluker and Beardsley 1970).

Pheidole megacephala and *L. humile* similarly showed their most defensive behavior when paired against *W. auropunctata* suggesting that this species may be a threat to both *P. megacephala* and *L. humile* if they are sympatric. *Wasmannia auropunctata* on the other hand was the least threatened by *A. gracilipes* showing the highest frequency of non-responsive acts in individual paired trials. Although *W. auropunctata* did not rank first in the aggression category, this species still elicited high levels of agonism shown in paired trails with *P. megacephala* and *L. humile*. Furthermore, this species had the highest overall frequency of biting behavior compared to all the species, as well as highest agonistic behavior from *P. megacephala* and *L. humile* in individual species paired trials. Based on our results it can be concluded that this new invader is a competitive threat to *P. megacephala* and *L. humile* thus influencing the dominance of these two species.

Using these individual and group laboratory assays as a tool to understand behavioral mechanisms that may have lead to the dominance of the four species tested, we would conclude that the newest invader, *W. auropunctata*, is the most likely candidate to become the next highly abundant, dominant, and destructive species present in Hawai'i. Looking only at the results from individual and group assays, it is not obvious that *W.*

auropunctata would be the likely dominant species compared to the other species. But, extrapolating the information taken from previous studies on these species in conjunction with our assays results, we can predict that *W. auropunctata* has the greatest potential to become Hawai'i's next dominant ant species. Although *P. megacephala* and *L. humile* were more aggressive in individual assays, they still showed their most defensive behavior when paired with *W. auropunctata*, as well as suffered high mortality in group assays when paired against *W. auropunctata*. *Anoplolepis gracilipes* had the highest average survival in group assays, but this species is restricted to particular nesting sites in the field which likely limits its expansion. Shown in other studies involving the invasion of *W. auropunctata*, this species is known to be extremely successful, as well as extremely detrimental to the native ecosystems it invades (Le Breton et al. 2005, Le Breton et al. 2004, Le Breton et al 2003). Other reports on the invasion biology of *W. auropunctata* on island ecosystems such as on New Caledonia confirm the potential for a widespread invasion (Le Breton et al. 2005, Le Breton et al. 2004, Le Breton et al. 2003, Wetterer and Porter 2003). Our laboratory assays force these four tramp ant species to interact, and we can thus extrapolate from the trials both on an individual and colony level which species are the most aggressive in interactions that otherwise may not have been revealed in the field.

CHAPTER 4 Ant Occurrences in Four Habitats in Hawai'i

Introduction

The Hawai'ian Islands are one of the few ecosystems in the world that lack native ants (Wilson and Taylor 1967, Reimer et al. 1990). Approximately 45 ant species have invaded Hawai'i, and about half of these are prominent in urban, agricultural and natural environments (Reimer 1994, Krushelnycky 2005). Since ant assemblages in Hawai'i are all synthetic, it is a unique and interesting environment in which to study invasion biology with respect to establishment, ecological interactions, and impacts.

In the present study, I explored the parallels between aggressive behavior in agonistic laboratory assays and abundance and dominance in the field. The four sites selected for field surveys are the same locations where we previously collected ant species for laboratory bioassays to examine interspecific behavioral interactions. These field sites are Waimanalo and Tantalus on the Island of Oahu, and Papaiko and Puu Huluhulu on the Island of Hawai'i. I propose that the species with highest levels of aggression would be the most abundant at each site from which they were collected in the previous year.

To summarize my laboratory agonism assays (see Chapters Two and Three). The first experiment conducted involved the four ant species *Leptogenys falcigera* Roger, *Camponotus variegatus* Smith, *Anoplolepis gracilipes* Smith, and *Wasmannia auropunctata* Roger in agonistic trials with one termite species, *Coptotermes formosanus* Shiraki. These ants were collected from Waimanalo, Tantalus, and Papaiko. The assays rated the first two interactions of individual ants and termites as either aggressive, no response or avoidance. The results demonstrated that *W. auropunctata* was the most

aggressive species towards the termite, while the other four species were largely non-responsive.

In the second laboratory experiment, I assessed agonistic interactions of the five ant species, *Leptogenys falcigera*, *Camponotus variegatus*, *Anoplolepis gracilipes*, *Ochetellus glaber* (Mayr, 1862), and *Pheidole megacephala* (Fabricius, 1793); all which were collected from two habitats at either Waimanalo or Tantalus. The assays conducted included individual and group trials to assess behavior on individual and colony levels. Accordingly to the results from individual and group assays *P. megacephala* and *A. gracilipes* were the most agonistic species.

In the third laboratory experiment, I used the four species, *P. megacephala*, *A. gracilipes*, *Linepithema humile* (Mayr), and *W. auropunctata*, thought to be dominant species in Hawai'i (Reimer et al. 1990, Reimer 1994, Krushelnycky 2005) and collected from Waimanalo, Tantalus, Papaiko, and Puu Huluhulu. Using the same protocol as the second experiment with individual and group assays, *Pheidole megacephala* and *L. humile* were ranked the most aggressive, respectably, in the individual assays. These two species showed the most defensive behavior when paired with *W. auropunctata*, as well as suffered high mortality in group assays paired against *W. auropunctata*. *Wasmannia auropunctata* appeared to be the species with the highest level of agonism when assessing specific species-paired trials in both individual and group assays.

The results from these series of agonistic laboratory assays indicate that the following species are the most aggressive, and therefore expected to be the most abundant species at their respective field sites; *P. megacephala* at Waimanalo, *A.*

gracilipes at Tantalus, *L. humile* at Puu Huluhulu, and *W. auropunctata* at Papaiko.

Thus, a field survey was initiated to substantiate these observations.

Methods and Materials

Site Descriptions

The two sites on the Island of Oahu differ slightly in elevation and temperature. *Anoplolepis gracilipes* was collected from Tantalus, which is 634 meters above sea level with an average yearly rainfall of 2662.5 mm. The other site at Waimanalo Research Station is 18m above sea level, with an average yearly rainfall of 1107.6mm and an average temperature of 23.8 degrees Celsius. We collected *P. megacephala*, *L. falcigera*, *C. variegatus*, and *O. glaber* from Waimanalo. We collected *L. humile* from Puu Huluhulu from the Island of Hawai'i. This collection site is in a National park protecting a "kipuka", a raised vegetated area surrounded by a younger lava flow. *Wasmannia auropunctata* was collected from Papaiko, a small town outside of the Hilo on the Island of Hawai'i. The site was on a small fruit orchard with mangosteen and rambutan trees.

Field surveys

Two hours was spent surveying each site. Baits (considered to be site "subsamples") consisting of *Starkist* tuna in oil, *Jiffy* peanut butter, and *Walkers* shortbread cookies were randomly placed on the ground or on tree trunks to sample the ant fauna throughout each site. The subsamples were checked every five minutes for the first hour and every twenty minutes for the remaining hour. All ant species were identified and photos were taken at every interval for accurate counts of individuals. Temperature and time were recorded at each site.

Results and Discussion

The results of the field survey were in agreement with our hypothesis at Waimanalo, Puu Huluhulu, and Papaiko, but not at Tantalus. *Pheidole megacephala* was the most abundant species at Waimanalo with 6,529 individuals (Table 1). The other species present at Waimanalo were *O. glaber* with 986 individuals and *Plagiolepis alluaudi* Emery with 556 individuals. *Linepithema humile* was the only species present at the baits in Puu Huluhulu with 370 individuals. *Wasmannia auropunctata* was the only species at the Papaiko site with the highest recorded number of individuals (13,467) out of all the species at all sites.

Anoplolepis gracilipes did not have highest abundance at Tantalus, where *Technomyrmex albipes* (Fr. Smith) was the most abundant species (Table 1). *Anoplolepis gracilipes* had the lowest abundance (15 individuals) compared to all species at all sites (Table 1). Although this species showed high levels of agonism in my laboratory assays, it was the least aggressive compared to *P. megacephala*, *L. humile* and *W. auropunctata*. This lower aggression (among aggressive species) may be the reason why this species did not fit our hypothesis, or *A. gracilipes* may not have been attracted to the baits provided in this study.

There appeared to be a change in ant fauna at Waimanalo and Tantalus over the period of ca. one year since our previous collections (Table 2). I did not observe any foraging workers of *P. alluaudi* previously, whereas currently it was one of the three species attracted to the baits at Waimanalo. The same is true at Tantalus in that *T. albipes* was not previously observed, but was recorded as the most abundant species present at the baits in the current survey. Thus we can conclude that agonistic assays

provide a generally realistic picture of ant species dominance in the field, but that community composition certainly may change over time. Colony size, occurrence of preferred microhabitats, and specific foraging preferences are other factors affecting ant community composition that are difficult to dissect in behavioral assays alone.

APPENDIX A: TABLES

Table 1.1. Percent mean comparing behavioral response among all four ant species responding to *C. formosanus* soldiers. To test overall species behavior using GLM Procedure (SAS 9.1), the percent mean response from the ant perspective was calculated and transformed to arcsine square root.

Ant Response	Avoid	No response	Aggressive
<i>L. falcigera</i>	11 ± 0.04 ^a	87 ± 0.06 ^a	2 ± 0.02 ^a
<i>C. variegatus</i>	2 ± 0.02 ^a	90 ± 0.06 ^a	8 ± 0.04 ^a
<i>A. gracilipes</i>	5 ± 0.03 ^a	82 ± 0.11 ^a	13 ± 0.09 ^a
<i>W. auropunctata</i>	3 ± 0.02 ^a	38 ± 0.06 ^b	59 ± 0.04 ^b

*Percentage in the same column followed by the same letter are not significantly different (p>0.05)

Table 1.2. Percent mean comparing behavioral response of *C. formosanus* soldiers responding to four ant species. To test overall species behavior using GLM Procedure (SAS 9.1), the percent mean response from the termite perspective was calculated and transformed to arcsine square root.

Termite Response	Avoid	No response	Aggressive
<i>L. falcigera</i>	0 ± 0.00 ^a	27 ± 0.14 ^a	73 ± 0.14 ^a
<i>C. variegatus</i>	0 ± 0.00 ^a	23 ± 0.16 ^a	77 ± 0.16 ^a
<i>A. gracilipes</i>	0 ± 0.00 ^a	48 ± 0.04 ^a	52 ± 0.04 ^{ab}
<i>W. auropunctata</i>	8 ± 0.06 ^a	73 ± 0.04 ^a	19 ± 0.07 ^b

*Percentage in the same column followed by the same letter are not significantly different (p>0.05)

Table 1.3. Behavioral response within each pair from termite and ant perspectives. GLM Procedure was used to test mean differences in behavioral responses within each pairing.

Ant Response		Termite Response	
1) <i>L. falcigera</i> vs. <i>C. formosanus</i>		1) <i>C. formosanus</i> vs. <i>L. falcigera</i>	
<u>Behavior</u>	<u>Percent mean</u>	<u>Behavior</u>	<u>Percent mean</u>
Avoid	11 ± 4.41a	Avoid	0 ± 0 a
No response	87 ± 6.01b	No response	27 ± 14.2 a
Aggression	2 ± 1.67a	Aggression	73 ± 14.2 b
2) <i>C. variegatus</i> vs. <i>C. formosanus</i>		2) <i>C. formosanus</i> vs. <i>C. variegatus</i>	
<u>Behavior</u>	<u>Percent mean</u>	<u>Behavior</u>	<u>Percent mean</u>
Avoid	2 ± 1.67 a	Avoid	0 ± 0 a
No response	90 ± 5.77 b	No response	23 ± 16.4 a
Aggression	8 ± 4.41 a	Aggression	77 ± 16.4 b
3) <i>A. gracilipes</i> vs. <i>C. formosanus</i>		3) <i>C. formosanus</i> vs. <i>A. gracilipes</i>	
<u>Behavior</u>	<u>Percent mean</u>	<u>Behavior</u>	<u>Percent mean</u>
Avoid	5 ± 2.89 a	Avoid	0 ± 0 a
No response	82 ± 10.9 b	No response	48 ± 4.41 b
Aggression	13 ± 8.82 a	Aggression	52 ± 4.41 b
4) <i>W. aurouunctata</i> vs. <i>C. formosanus</i>		4) <i>C. formosanus</i> vs. <i>W. auropunctata</i>	
<u>Behavior</u>	<u>Percent mean</u>	<u>Behavior</u>	<u>Percent mean</u>
Avoid	33 ± 30.9 a	Avoid	8 ± 6.01 a
No response	38 ± 6.01 b	No response	73 ± 4.41 b
Aggression	59 ± 4.41 c	Aggression	18 ± 7.26 a

*Percentage in the same column followed by the same letter are not significantly different (p > 0.05)

Table 2.1. Individual assays: Total behavioral interactions for each species paired with the four other species. Nine behaviors were accounted for ranging from aggressive to defensive behavior from left to right in the table. GLM Procedure was used to test mean differences among nine behavioral responses exclusively for each species.

Species	BT	GF	AT	MO	IN	MI	ES	BA	BB
<i>Camponotus variegatus</i>	8 ^a	5 ^a	52 ^{ab}	62 ^{abc}	29 ^{ab}	123 ^c	75 ^{b^c}	45 ^{ab}	5 ^a
<i>Anoplolepis gracilipes</i>	6 ^a	9 ^a	70 ^{acd}	73 ^{cd}	38 ^{abc}	101 ^d	71 ^{acd}	27 ^{ab}	3 ^a
<i>Ochetellus glaber</i>	4 ^a	51 ^a	18 ^a	41 ^a	43 ^a	108 ^b	41 ^a	16 ^a	9 ^a
<i>Pheidole megacephala</i>	5 ^a	0 ^a	21 ^{ab}	180 ^c	45 ^{ab}	74 ^b	11 ^a	11 ^a	0 ^a
<i>Leptogenys falcigera</i>	1 ^a	0 ^a	1 ^a	3 ^a	157 ^c	87 ^b	29 ^a	18 ^a	2 ^a

(BT- bite, GF- gaster flex, AT- attack, MO- mandibles open, IN- indifference, MI mutual investigation, ES- escape, BA- being attacked, BB- being bitten).

*Values in the same row followed by the same letter are not significantly different (P > 0.050)

Table 2.2. Nine behavioral interactions listed in Table 1 overall species behavior paired against four other species) broken down into three categories of aggressive, no response, and defensive. The species were ranked within these categories, with 1 being the highest in the category.

Species	Aggression	No response	Defensive
<i>Camponotus variegatus</i>	4	3	1
<i>Anoplolepis gracilipes</i>	2	4	2
<i>Ochetellus glaber</i>	3	2	3
<i>Leptogenys falcigera</i>	5	1	4
<i>Pheidole megacephala</i>	1	5	5

(Bite, gaster flex, attack, mandibles open included in the aggressive category; indifference and mutual investigation are included in the no response category; escape, being attacked, and being bitten are included in the defensive category)

Table 2.3. Group assays: Average percent survival of individuals for each pairing, rows show average survival when that species was paired with the species listed in the column.

Species	<i>A. gracilipes</i>	<i>O. glaber</i>	<i>C. variegatus</i>	<i>P. megacephala</i>	<i>L. falcigera</i>
<i>A. gracilipes</i>	100%	85%	70%	70%	100%
<i>O. glaber</i>	63%	99%	90%	85%	100%
<i>C. variegatus</i>	93%	98%	100%	93%	100%
<i>P. megacephala</i>	0%	30%	8%	98%	100%
<i>L. falcigera</i>	100%	100%	100%	100%	100%

Average survival in controlled trials for each species is indicated in bold font.

Table 3.1. Individual assays: Total behavioral interaction for each species paired with the four other species. Nine behaviors were accounted for ranging from aggressive to defensive behavior from left to right in the table. GLM Procedure was used to test mean differences among nine behavioral responses exclusively for each species.

Species	BT	GF	AT	MO	IN	MI	ES	BA	BB
<i>Anoplolepis gracilipes</i>	0 ^a	13 ^a	4 ^a	18 ^a	57 ^b	68 ^b	47 ^b	9 ^a	2 ^a
<i>Pheidole megacephala</i>	6 ^{ab}	0 ^{ab}	23 ^a	89 ^b	12 ^{ab}	58 ^c	3 ^{ab}	12 ^{ab}	11 ^{ab}
<i>Linepithema humile</i>	0 ^a	7 ^a	11 ^{ab}	29 ^{bc}	6 ^a	46 ^c	7 ^a	10 ^{ab}	2 ^a
<i>Wasmannia auropunctata</i>	16 ^a	0 ^a	7 ^a	14 ^a	61 ^b	50 ^b	0 ^a	7 ^a	7 ^a

(BT- bite, GF- gaster flex, AT- attack, MO- mandibles open, IN- indifference, MI mutual investigation, ES- escape, BA- being attacked, BB- being bitten).

*Values in the same row followed by the same letter are not significantly different ($P > 0.050$)

Table 3.2. Nine behavioral interactions broken down into three categories of aggressive, no response, and defensive for each species pairing in individual assays.

***P. megacephala* response to other species**

<i>W. auropunctata</i>	<i>L. humile</i>	<i>A. gracilipes</i>
Aggressive-44	Aggressive-15	Aggressive-59
No response-28	No response-20	No response-22
Defensive-20	Defensive-5	Defensive-1

***L. humile* response to other species**

<i>W. auropunctata</i>	<i>P. megacephala</i>	<i>A. gracilipes</i>
Aggressive-2	Aggressive-21	Aggressive-24
No response-13	No response-19	No response-20
Defensive-10	Defensive-6	Defensive-3

***W. auropunctata* response to other species**

<i>L. humile</i>	<i>P. megacephala</i>	<i>A. gracilipes</i>
Aggressive-13	Aggressive-31	Aggressive-3
No response-11	No response-44	No response-56
Defensive-0	Defensive-14	Defensive-0

***A. gracilipes* response to other species**

<i>L. humile</i>	<i>W. auropunctata</i>	<i>P. megacephala</i>
Aggressive-10	Aggressive-14	Aggressive-11
No response-23	No response-62	No response-40
Defensive-19	Defensive-6	Defensive-33

(See Table 2 for specific behaviors within each category)

Table 3.3. The nine overall behavioral interactions listed in Table 1 broken down into three categories of aggressive, no response, and defensive. The species were ranked within these categories, with 1 being the highest in the category.

Species	Aggression	No response	Defensive
<i>Anoplolepis gracillipes</i>	4	2	1
<i>Pheidole megacephala</i>	1	4	3
<i>Linepithema humile</i>	2	3	2
<i>Wasmannia auropunctata</i>	3	1	4

(Bite, gaster flex, attack, mandibles open included in the aggressive category; indifference and mutual investigation are included in the no response category; escape, being attacked, and being bitten are included in the defensive category)

Table 3.4. Group assays without *P. megacephala* soldiers present: Average percent survival of individuals for each pairing, rows show average survival when that species was paired with the species listed in the column.

Species	Tot. average survival	<i>A. gracilipes</i>	<i>P. megacephala</i>	<i>L. humile</i>	<i>W. auropunctata</i>
<i>A. gracilipes</i>	77%	100%	78%	75%	78%
<i>P. megacephala</i>	11%	0%	95%	33%	0%
<i>L. humile</i>	35%	18%	85%	95%	3%
<i>W. auropunctata</i>	49%	43%	65 %	38%	95%

Average survival in control trials for each species is indicated in bold font.

Table 3.5. Group assays without *P. megacephala* soldiers present: Average percent survival of individuals for each pairing, rows show average survival when that species was paired with the species listed in the column.

Species	Tot. average survival	<i>A. gracilipes</i>	<i>P. megacephala</i>	<i>L. humile</i>	<i>W. auropunctata</i>
<i>A. gracilipes</i>	83%	100%	95%	75%	78%
<i>P. megacephala</i>	36%	55%	95%	15%	38%
<i>L. humile</i>	12%	18%	15%	95%	3%
<i>W. auropunctata</i>	42%	43%	45 %	38%	95%

Average survival in control trials for each species is indicated in bold font.

Table 4.1. Ant species and numbers of individuals present at four locations on the Islands of Oahu and Hawai'i.

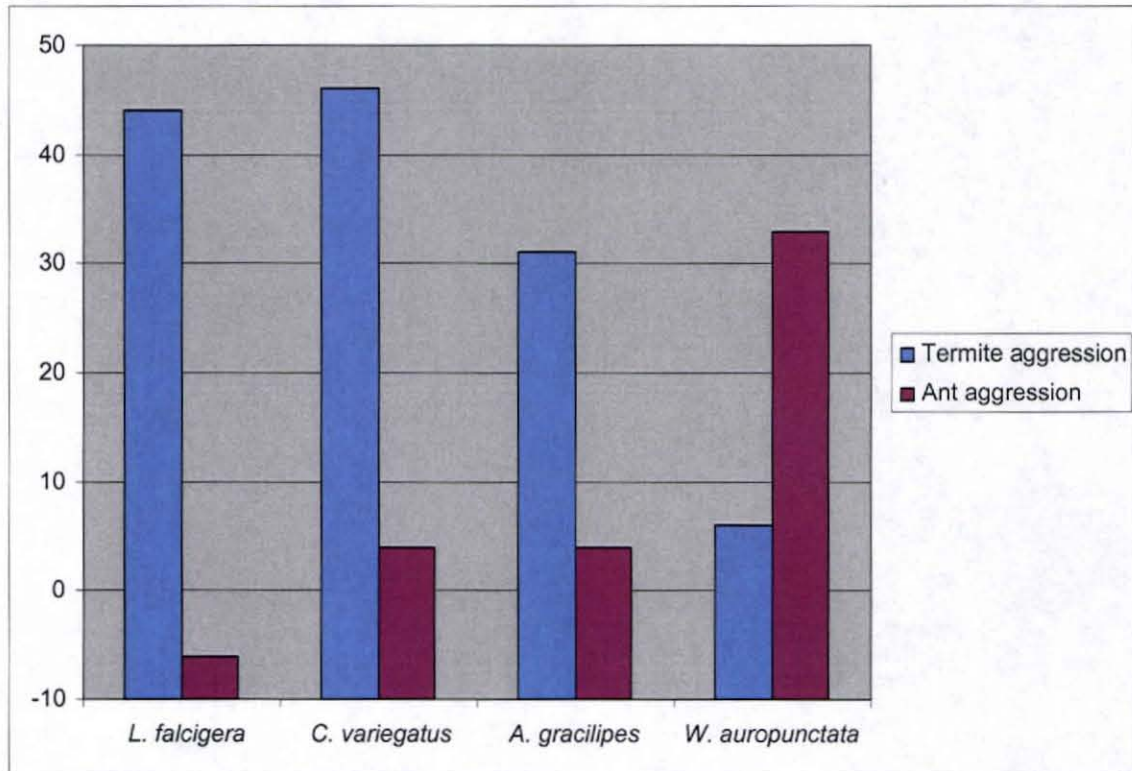
Waimanalo (Oahu)	
Species	Total present
<i>Pheidole megacephala</i>	6,529
<i>Plagiolepis alluandi</i>	556
<i>Ochetellus glaber</i>	986
Tantalus (Oahu)	
Species	Total present
<i>Anoplolepis gracilipes</i>	15
<i>Technomyrmex albipes</i>	256
Puu Huluhulu (Hawaii)	
Species	Total Present
<i>Linepithema humile</i>	370
Papaiko (Hawaii)	
Species	Total present
<i>Wasmannia auropunctata</i>	13,467

Table 4.2. Ant species collected at each field site in 2005, and the species present on baits in the current survey approximately 1 year later (2006).

Waimanalo	2005	2006
<i>Pheidole megacephala</i>	X	X
<i>Plagiolepis alluaudi</i>		X
<i>Ochetellus glaber</i>	X	X
<i>Camponotus variegatus</i>	X	
<i>Leptogenys falcigera</i>	X	
Tantalus		
<i>Anoplolepis gracilipes</i>	X	X
<i>Technomyrmex albipes</i>		X
Puu Huluhulu		
<i>Linepithema humile</i>	X	X
Papaiko		
<i>Wasmannia auropunctata</i>	X	X

APPENDIX B: FIGURES

Figure 1.1 Totaled behavioral response from each species in agonistic assays. Levels of aggressive behavior are indicated with higher bars.



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